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Comparative karyotype analysis of three *Passiflora* L. species and cytogenetic characterization of somatic hybrids

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Abstract — The present study was carried out to analyze the karyotypes of three *Passiflora* species with 2n = 18 (the yellow passion fruit *P. edulis* f. *flavicarpa* and the wild species *P. amethystina* and *P. cincinnata*) previously used to obtain somatic hybrids by protoplast fusion. A biometrical analysis of Feulgen stained metaphases showed that the karyotypes of these species were quite similar with subtle differences in the morphology of some chromosomes between the species. These data were integrated with the physical mapping of the 45S rDNA (18S-5.8S-26S rRNA genes) and 5S rDNA loci using FISH, and with the pattern of fluorescent staining with chromomycin A_3. FISH signals of 45S rDNA were localized on the secondary constriction and satellite of two chromosome pairs, identified as 8 and 9, in the three species. An additional site was observed on the long arm of chromosome 1 in *P. amethystina*. Chromomycin A_3 staining revealed highly fluorescent bands corresponding to 45S rDNA loci. One 5S rDNA site was detected on the long arm of chromosome pair 4 in the three species. Silver staining showed four positive signals on the small satellited chromosomes, and the locus on chromosome 1 of *P. amethystina* seemed to be inactive. Somaclones derived from four somatic hybrid plants (*P. edulis* + *P. amethystina*) had 2n = 4x = 36, but in samples of two hybrids, 2n = 35 was observed. Variability in the number of 45S rDNA sites (10 or 12) was detected among the hybrids, and the FISH signals were smaller than in the parental species. These findings suggest that DNA sequence loss and transposition occurred in these newly formed polyploids.

Key words: FISH; fluorochrome banding; karyotype characterization; *Passiflora*; rDNA sites; somatic hybrids.

INTRODUCTION

The genus *Passiflora* L. (Passifloraceae), that includes the passion fruit, is a large and widespread genus consisting primarily of tropical species. It comprises approximately 465 species of vines, though some representatives are shrubs or trees. South America is the centre of diversity for most of the *Passiflora* species; around 40 are indigenous to Asia and South Pacific Islands. Passion vines are evergreen climbers, grown for their edible fruits, and several species are cultivated for their unusual and beautiful flowers (Vanderplank 1996).

Among all, about 50 species bear edible fruits but only the two forms of *Passiflora edulis, i.e.*, the purple and the yellow ones are considered to be of value in international commerce. The wide genetic variability, known to exist within and between *Passiflora* species can be exploited in breeding programs. It is of interest to establish the mapping of genes for agronomic traits and resistance to pests and diseases in *P. edulis*. Interspecific sexual hybridization has been attempted, but the hybrids were difficult to be obtained and showed low fertility (Oliveira and Ferreira 1991). In order to produce rootstocks resistant to soil-borne diseases, interspecific protoplast fusion was performed, and the hybrids *P. edulis* + *P. amethystina* and *P. edulis* + *P. cincinnata* were obtained (Dornelas *et al.* 1995; Vieira and Dornelas 1996).

A standardization of the karyotypes of *Passiflora* species will be extremely important, not only to understand the evolution of the genus, but also to assist breeding programs. The chromosome numbers of approximately 100 species of *Passiflora* have been reported, and the most frequent are 2n = 2x = 12, 2n = 2x = 18 and 2n = 2x =20, but some polyploids (2n = 24, 2n = 36 and 2n = 72) have also been described (Bowden 1945; Storey 1950; Beal 1971; 1973a, b; Snow and MacDougal 1993; Melo *et al.* 2001; Melo and Guerra 2003). The base number has been dis-

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cussed, and recently, MELO and GUERRA (2003) investigated the variability of 5S and 45S rDNA sites, concluding that the results were consistent with the hypothesis of x = 6 as the base number, while species with x = 9, 10 and 12 would be of tetraploid origin, with descending dysploidy. Most of these studies aimed at the determination of the chromosome numbers of *Passiflora* species and have shown that they have symmetrical karyotypes, with a variable number of satellites. A comparative karyotype analysis has not been made, except for a study of six Brazilian species (2n = 18), based on conventional staining (SOUZA *et al.* 2003).

The use of molecular cytogenetic methods, based on fluorescent staining and *in situ* hybridization of repetitive DNA sequences, has provided valuable markers for chromosome identification and studies on genome organization and evolution of several plant genera, including species of agronomic interest, such as *Vigna* (GALASSO *et al.* 1995), *Hordeum* (LEITCH and HESLOP-HARRISON 1993; TAKETA *et al.* 2000), *Triticum* (ARAGON-ALCAIDE *et al.* 1996; CUADRADO and SCHWARZACHER 1998; KISHII and TSUJIMOTO 2002) and *Nicotiana* (KENTON *et al.* 1993; LIM *et al.* 2000).

The main commercial species of passion fruit have a chromosome number of 2n = 18. It is of interest to identify the individual chromosomes and to standardize the karyotype of *P. edulis*, as linkage maps were recently established (CARNEIRO *et al.* 2002; LOPES 2003), and a correlation between both genetic and physical maps is highly desirable.

We carried out the present study with the purpose of analyzing the karyotypes of three species previously used to produce somatic hybrids, namely, *P. amethystina, P. edulis* f. *flavicarpa* and *P. cincinnata.* Conventional staining and molecular cytogenetic methods were employed to examine the morphology of the chromosomes, to detect the pattern of fluorescent banding using chromomycin A_3, and to map the sites of 45S rDNA (18S-5.8S-26S rRNA genes) and 5S rDNA using fluorescent *in situ* hybridization (FISH), besides testing NOR activity by silver nitrate staining. Somatic hybrids (*P. edulis* f. *flavicarpa* + *P. amethystina*) were also examined, to address their chromosome stability.

MATERIAL AND METHODS

Plant material - The species *P. amethystina* Mikan (subgenus *Passiflora,* Series *Lobatae*), *P. edulis* Sims f. *flavicarpa* Degener (subgenus *Passiflora,* Series *Passiflora*), the yellow passion fruit, and *P. cincinnata* Mast. (subgenus *Passiflora,* Series *Passiflora*) were studied. KILLIP's classification (1938) was adopted. The meiotic behaviour of somatic hybrids between *P. edulis* f. *flavicarpa* and *P. amethystina* (referred to as E + Am # 12, E + Am # 13, E + Am # 28, E + Am # 35) has been previously investigated (BARBOSA and VIEIRA 1997), and samples of somaclones derived from these materials were analyzed in the present work.

Chromosome preparation - All plants were propagated vegetatively as stem cuttings, from which roots were obtained, pretreated in 8-hydroxyquinoline at 300 ppm combined with cycloheximide at 3.125 ppm for 90 min, and fixed in ethanol-acetic acid (3:1), as previously described (CUCO *et al.* 2003). Roots of about 20 cuttings from each somatic hybrid were used.

For karyotype analysis, root tips were stained by the Feulgen method and digested in an enzyme solution (2% cellulase [w/v] and 3% [v/v] pectinase in citrate buffer) at 37°C for 13 min, just before squashing in 45% acetic acid. Chromosome arms were measured in ten metaphase spreads of each species and photographed with Kodak Technical Pan film (ISO 25). Relative chromosome lengths were expressed as percentage of the haploid set, and chromosomes were classified according to their arm ratio, as described by LEVAN *et al.* (1964).

For chromomycin A_3/ distamycin (CMA/DA) staining and FISH, roots were squashed in 60% acetic acid, coverslips were removed in liquid nitrogen, and the slides maintained at –20°C.

DNA probe labelling and in situ hybridization - The 45S rDNA probe used was the 9.1 kb repeating unit of maize rDNA (MCMULLEN *et al.* 1986) cloned in pUC8 at *Eco*RI. The 5S rDNA probe was amplified from total genomic DNA of *P. edulis* by PCR using the primer pair: 5'GTGCGATCATACCAGC (AG)(CT)TAATGCACCGG3' and 5'GAGGTGCAACACGAGGACTTCCC AGGAGG3', designed according to the 5SrDNA sequence of *Glycine* (GOTTLOB-MCHUGH *et al.* 1990). Probes were labelled with biotin-14-dATP by nick translation, using a Bionick Labelling System Kit (Gibco BRL), or with digoxigenin-11-dUTP by random primer labelling (Roche).

The FISH procedure was based on SCHWARZACHER and HESLOP-HARRISON (2000), with slight modifications. Briefly, preparations were pretreated with DNase-free RNase (100 µg/ml in 2 x SSC), for 1h, and pepsin (5 µg/ml in 10

mM HCl), for 30 min, both at 37°C, and post-fixed in 4% paraformaldehyde in water for 10 min, dehydrated in an ethanol series, and air dried. The hybridization mixture contained 8 ng/µl of DNA probe, and after overnight hybridization at 37°C, the slides were washed in 0.5 x SSC for 5 min, 40% formamide for 10 min, and 2 x SSC for 5 min, at 42°C. Biotin-labelled probes were detected using the mouse anti-biotin antibody, followed by FITC-conjugated rabbit anti-mouse (Dako), and the detection of digoxigenin-labelled probes was done with fluorescein- or rhodamine-conjugated sheep anti-digoxigenin antibody (Roche). Preparations were counterstained with propidium iodide (1.6 µg/ml) or 4',6-diamidino-2-phenylindole (DAPI) (10 µg/ml), and mounted in Vectashield H1000 (Vector). Chromosome spreads were analyzed under a Zeiss Axiophot-2 epifluorescence microscope with appropriate filters. The images were captured with a CCD video camera, using the ISIS software (Meta Systems) and printed in a thermal colour printer.

Chromomycin A_3/Distamycin and silver staining - Chromomycin A_3 staining in combination with distamycin A was based on FRIEBE *et al.* (1996). 45S rDNA transcriptional activity was checked by silver staining, following the method of HOWELL & BLACK (1980).

RESULTS

Karyotype analysis - The karyotypes of the three *Passiflora* species (2n = 18) are illustrated in Fig. 1 and the respective idiograms are shown in Fig. 2. They are symmetric and quite similar, as can also be seen in Table 1, where chromosome lengths and arm ratios are presented. The haploid set

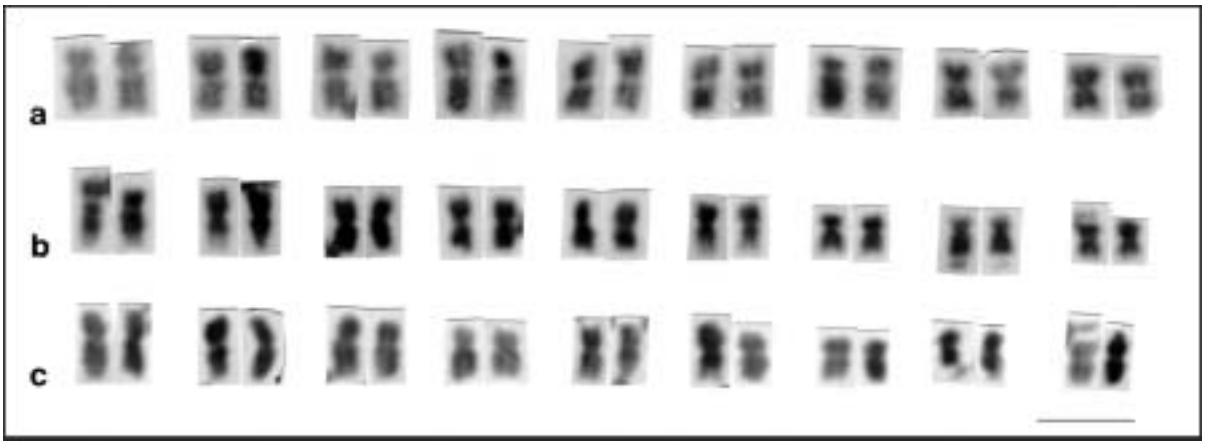

Fig. 1 — Karyotypes of *Passiflora amethystina* (a), *P. edulis* f. *flavicarpa* (b) and *P. cincinnata* (c), showing 2n = 18 chromosomes and the presence of secondary constrictions and satellites on chromosomes 8 and 9. Due to chromosome condensation in *P. amethystina*, the satellites are not clearly seen. Bar = 5 µm.

Table 1 — Chromosome lengths and arm ratios of *Passiflora amethystina*, *P. edulis* f. *flavicarpa* and *P. cincinnata*

Species	Chromosome									Haploid length (µm)
	1	2	3	4	5	6	7	8	9	
Chromosome length (µm)										
P. amethystina	3.23	3.23	3.14	3.14	2.91	2.64	2.62	2.42	2.35	25.68
P. edulis	3.16	3.00	2.76	2.79	2.60	2.36	2.15	2.02	1.82	22.66
P. cincinnata	2.77	2.70	2.49	2.19	2.20	2.00	1.87	1.64	1.79	19.65
Relative length (%)										
P. amethystina	12.58	12.58	12.23	12.23	11.33	10.28	10.20	9.42	9.15	
P. edulis	13.95	13.24	12.18	12.31	11.47	10.41	9.49	8.92	8.03	
P. cincinnata	14.09	13.75	12.68	11.14	11.20	10.18	9.51	8.35	9.10	
Arm-ratio										
P. amethystina	1.81	1.14	1.20	1.55	1.17	1.18	1.61	1.32	1.11	
P. edulis	1.84	1.14	1.16	1.33	1.26	1.21	1.30	1.53	1.16	
P. cincinnata	1.58	1.21	1.26	1.81	1.21	1.30	1.43	1.26	1.32	

length varied from 25.68 µm in *P. amethystina* to 19.65 µm in *P. cincinnata,* and most chromosomes were metacentric. Chromosome 1 was submetacentric and the pair identified as chromosome 4 could be recognized by its arm ratio, that was rather higher in *P. amethystina* and *P. edulis* (Fig. 2a and 2b), whereas in *P. cincinatta* (Fig. 2c) it was clearly submetacentric. Also, chromosome 7 had a submedian centromere in *P. amethystina*. Secondary constrictions and satellites were observed on the smallest chromosomes of the three species, i.e., on the long arm of chromosome 8 (8L) and on the short arm of chromosome 9 (9S). The size and arm ratio of these chromosomes were estimated without including the length of the satellite and secondary constriction, in view of their variable degree of stretching in the metaphase spreads. The presence of satellites on the above mentioned arms, was adopted as the main criterion to classify these chromosomes as pairs 8 and 9, even in the case of *P. cincinnata*, in which the pair bearing a satellite on the short arm (identified as chromosome 9) was larger than the pair classified as number 8. In Fig. 1a, due to the degree of chromosome condensation, the satellites of *P. amethystina* are not as clearly seen as they are, in less condensed metaphases (see Fig. 3h).

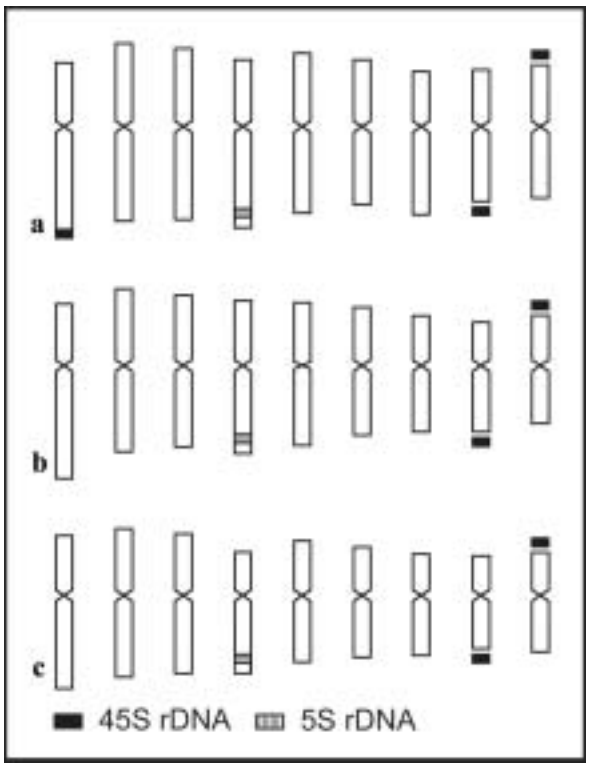

Fig. 2 — Idiograms of *P. amethystina* (a), *P. edulis* f.*flavicarpa* (b) and *P. cincinnata* (c) showing the localization of 45S and 5S rDNA sites.

Physical mapping of 45S and 5S rDNA sites - Fluorescent *in situ* hybridization using the 45S rDNA probe showed the presence of six sites in *P. amethystina* and of four in *P. edulis* and *P. cincinnata* (Figs. 2 and 3 a-c). In all the three species, the hybridization signals were detected on the secondary constrictions and satellites of chromosomes 8 and 9. In *P. amethystina*, an additional site was observed at a terminal position on the long arm of chromosome 1, in which no secondary constriction was observed (Figs. 2a and 3a). *In situ* hybridization using the 5S rDNA probe detected a single site at a subterminal position on the long arm of chromosome 4 in all the three species analyzed (Figs. 2 and 3h-j).

Chromomycin A_3 banding and silver staining - CMA positive bands were found in regions corresponding to 45S rDNA sites, therefore, in three pairs of chromosomes in *P. amethystina* and in two pairs in *P. edulis* and *P. cincinnata* (Fig. 3d-f). No other preferentially GC-rich regions were observed in these species.

Nucleolar activity investigated by silver staining revealed four silver-positive signals on the small satellited chromosomes in the three species (Fig. 3g). The 45S rDNA site located on chromosome 1 of *P. amethystina* seems to be inactive, as no corresponding silver signal (not shown) or secondary constriction was found on this chromosome.

Cytogenetic characterization of the somatic hybrids - All the previously investigated somatic hybrids E + Am and E + C had 2n = 4x = 36 (Vieira and Dornelas, 1996; Barbosa and Vieira 1997). However, in the present investigation, 35 chromosomes were observed in the samples of E + Am # 12 and E + Am # 28. The lack of one chromosome did not affect the vegetative development and flowering of the plants. Conversely, both the hybrids E + Am and E + C did not set normal fruits, even when artificially crossed (Barbosa and Vieira 1997).

As expected, ten 45S rDNA sites were detected by FISH in the somatic hybrids E + Am # 12 (Fig 3k and 3l) and E + Am # 28, whereas the hybrids E + Am # 13 (Fig. 3m) and E + Am # 35 showed 12 sites. At first, we supposed that these two particular somaclones were not interspecific hybrids, and had resulted from the fusion of two *P. amethystina* protoplasts. If so, two pairs of large

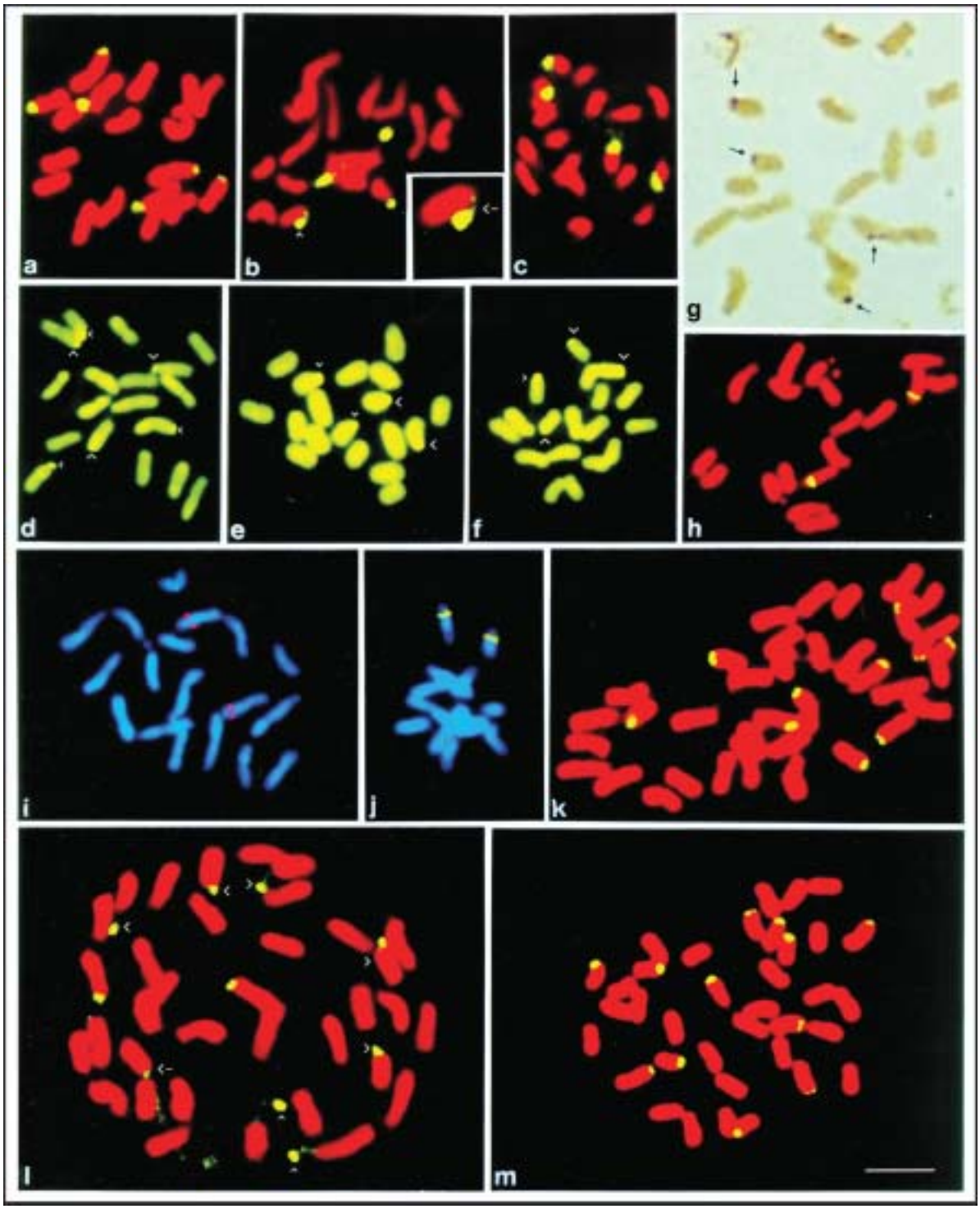

Fig. 3 — Mitotic chromosomes of the *Passiflora* species: *P. amethystina* (a, d, h), *P. edulis* f *flavicarpa* (b, e, g, i), *C. cincinnata* (c, f, j). 45S rDNA loci revealed by FISH (a, b, c); note an amplified chromosome (arrow) in (b), showing the signal on the secondary constriction and satellite. CMA/DA fluorescent bands corresponding to these sites (d, e, f). Silver staining showing four active NORs (arrows) in a metaphase of *P. edulis* (g). 5S rDNA sites detected by FISH in a chromosome pair identified as pair 4 in the three species (h, i, j). 45S rDNA sites in the somatic hybrids (k, l, m). Metaphase cell showing ten sites in E + Am # 12 (2n = 35) (k) and 12 sites in E + Am # 13 (2n = 36) (m). Note the presence of 7 satellites (arrowheads) and an uncondensed terminal secondary constriction (arrow) in a prometaphase of hybrid E + Am # 12; a large chromosome (pair 1) shows an unusual secondary constriction and satellite (l). FISH preparations are counterstained with propidium iodide (a, b, c, h, k, l, m) or DAPI (i, j). Bar = 5 um.

chromosomes (pair 1) would be expected to show signals, but this was not observed. Another kind of event may have occurred, as discussed below. Furthermore, in most metaphase spreads analyzed, the signals were smaller in comparison with those visualized in the parental metaphases, and this also suggests that alterations may have occurred, either *in vitro* or as a consequence of interspecific hybridization.

Silver staining revealed at least eight signals in a sample of the hybrid E + Am # 12 (not shown). Also, about 7-8 chromosomes exhibiting a secondary constriction and satellite could be visualized in prophases and prometaphases, in which at least one small chromosome did not show a satellite but an uncondensed terminal secondary constriction, as seen in a prometaphase of hybrid E + Am # 12 (Fig. 3l), in which an unusual secondary constriction and satellite appear in one large chromosome (pair 1). These observations suggest that a variable number of rDNA sites may be active in these hybrids, and are of interest for a further cytogenetic control of a larger number of cuttings derived from the hybrids E + Am, as discussed below.

DISCUSSION

Passiflora species - The three *Passiflora* species investigated are closely related as they belong to the same subgenus (*Passiflora*), although *P. amethystina* belongs to the series *Lobatae*, and *P. edulis* and *P. cincinnata* are members of the series *Passiflora.* They have symmetrical karyotypes (2n = 18), which could be distinguished from each other, by minor differences. MELO *et al.* (2001), who analyzed the chromosome numbers of 31 species of *Passiflora* species distributed through different subgenera, did not describe the karyotypes, but showed that they were symmetrical. Furthermore, MELO and GUERRA (2003) reported that chromosome size asymmetry was observed only in the group of species with x = 6. In the three species analyzed, we detected secondary constrictions at a sub-terminal position on the long and short arms, respectively, of the chromosomes identified as pairs 8 and 9, and our observations are quite consistent with the finding of two pairs of small chromosomes bearing positive silver signals, thus suggesting that they have active rDNA sites.

As for the fluorochrome banding patterns, the use of chromomycin revealed pale bands coincident with the 45S rDNA sites, while, when the CMA/DA combination was used, these regions appeared very bright in the three species. It is interesting to note that the GC-specific antibiotic chromomycin A_3, used as fluorescent dye in combination with distamycin (AT-specific non-fluorescent drug) may produce a very bright fluorescent pattern, and these brilliant regions, which are resistant to quenching by counterstaining, have been interpreted as locations of DNA with many clusters of base pairs of the type for which the fluorescent stain is specific (see SCHWEIZER 1981). CMA/DA positive NORs have been detected in several species of plants, as for instance, in *Vicia faba* (FUCHS *et al*. 1998). Our results are in agreement with previous observations in *Passiflora* species (MELO *et al.* 2001), but these authors reported only five CMA+ blocks in *P. amethystina.*

In the current study, the 45S rDNA loci detected by FISH are in accordance with those reported by MELO and GUERRA (2003) concerning the number of sites, but differ in the description of the chromosomes carrying rDNA loci. This discrepancy may be due to our criterion of classification of the satellited chromosomes according to their length, estimated without considering the size of the satellites. We therefore assumed that these chromosomes are homoeologous in these three species, which, as mentioned above, are closely related and possibly have conservative karyotypes. This interpretation of homoeology of chromosomes with similar signals can be found in other studies, as, for instance, in the comparative karyotype analysis in the *Nicotiana* section Tomentosae (LIM *et al.* 2000). In the case of *P. amethystina*, the 45S rDNA sites were reported to be located on chromosomes 5, 6 and 9 (MELO and GUERRA 2003). Moreover, our observation of the 5S rDNA site on the long arm of a pair of chromosomes identified as number 4, differed from this previous report (MELO and GUERRA 2003) locating this site on different chromosomes, in the three species.

In a study on 20 *Passiflora* species (x = 6, 9, 10 and 12), in which a description of the karyotypes was not reported (MELO and GUERRA 2003), the number of 5S rDNA sites was almost constant, usually a pair for each species, and the number and location of 45S rDNA sites were variable (one to three loci per haploid set). It is interesting to note that, in an analysis of the karyotypes of six Brazilian species with x = 9 (SOUZA *et al.* 2003), other than those described in our work, secondary constrictions and adjacent satellites were reported to be in variable positions of the small and/or large chromosomes. In fact, an accurate identifi-

cation of individual chromosomes in more *Passiflora* species should be done, for a better understanding of karyotype evolution in this genus.

Actually, variability in the number and location of clusters of rRNA genes has been found in several groups of plants, as for instance, in a study of seven *Oryza* species, in which the number of 45S rDNA loci ranged from one to three per haploid genome, but apparently a major locus was located on the same chromosome pair in most of the species examined (Shishido *et al.* 2000). The results were interpreted as evidence of the occurrence of transposition events, as also suggested previously for *Allium* (Schubert and Wobus 1985). Also, in *Hypochaeris* species with different chromosome numbers (x = 3, 4, 5, and 6), rearrangements and transposition of rDNA loci must have occurred during evolution (Cerbah *et al.* 1998).

Melo and Guerra (2003) suggested that the number and location of 5S and 45S rDNA sites were consistent with the hypothesis of x = 6 being the probable ancestral genome of the genus *Passiflora*, while the groups of species with x = 9, x = 10 and x = 12 would be of tetraploid origin, with descending dysploidy (12 ? 10 ?9) and reduction of redundant sites, mainly the 5S rDNA ones. Furthermore, only in two samples of the species *P. misera* and *P. tuberosa*, site duplication proportional to the ploidy level was found. As for the species investigated here, if we assume that they are polyploids originated, as proposed, from an ancestral with x = 6, we can speculate that in *P. amethystina*, transposition of rDNA sequences from a homoeologue of chromosome 8 or 9 to the largest chromosome could have occurred during evolution. Apparently this locus, in which a secondary constriction or a positive silver signal was not detected, is silent, whereas the loci on the smaller chromosomes 8 and 9 must have rDNA transcriptional activity in root cells of the three species investigated. Furthermore, a previous meiotic analysis of *P. amethystina* and *P.edulis* f. *flavicarpa*, showing two bivalents associated with the nucleolus in most cells examined (Barbosa and Vieira 1997), represents additional evidence for the presence of two pairs of chromosomes carrying active NORs in these species.

It is interesting to note that the present study allowed the standardization of the karyotypes of the three species investigated, based on the chromosome morphology and location of the 45S and 5S rDNA loci. These sites have been useful as landmarks for chromosome identification in the analysis of somatic hybrids, and as mentioned earlier, the characterization of the karyotype of *Passiflora edulis* is particularly important for allocating linkage groups to physical chromosome maps and, obviously, to contribute to evolutionary studies.

Cytogenetic analysis of somatic hybrids - The cytogenetic analysis of samples of the somatic hybrids E + Am carried out in this study provided interesting information on genetic alterations occurred in these newly formed polyploids. In recent years, there has been an increasing interest in studies aiming at understanding the genetic events involved in early allopolyploid formation. Mechanisms of chromosome diploidization enabling genome stability and fertility (provided by bivalent formation and proper chromosome segregation at meiosis), along with gene loss or inactivation (to deal with gene redundancy), allowed newly formed allopolyploids to establish themselves as successful species. Genetic events associated with allopolyploid evolution involving alterations in rDNA structure, content and activity have been shown to occur in natural and newly formed allopolyploids (for a review, see Leitch and Bennett 1997; Pikaard 1999; Eckardt 2001; Skalická *et al.* 2003).

The observation of smaller 45S rDNA signals in somaclones of the four E + Am hybrids analyzed suggests the occurrence of partial loss of these sequences, without complete elimination of any of these loci. It is interesting to consider that the observation of 7-8 NOR-chromosomes bearing secondary constrictions (as seen in Fig. 3l) suggests that the two pairs of chromosomes which are active in each parental species remain transcriptionally active in the root meristem cells of the hybrids. On the other hand, the presence of a secondary constriction in one large chromosome, observed in this same figure, suggests that one homologue of pair 1 may be active in this hybrid. These conclusions seem to be supported by the presence of at least eight silver signals revealed in some metaphases of the hybrid E + Am #12. This analysis should be extended to more samples of the hybrids E + Am, to investigate the degree of variability among plants that have been propagated vegetatively as stem cuttings. Furthermore, the presence of 12 loci of 45S rDNA in two hybrids (E+Am # 13 and E + Am # 35) may be due to the occurrence of extra loci possibly originated by transposition followed by amplification of the rDNA units. All these aspects reveal that these hybrids may be suffering modifications, as has been shown in many studies of newly formed allopoly-

ploids. Silencing of transcription of the rRNA genes of one of the parental sets (nucleolar dominance) in amphiploids has been observed in many organisms (see PIKAARD 1999). Rearrangements of the 45S rDNA have also been found in somatic hybrids, as for instance, between *Medicago* species (CLUSTER *et al*. 1996). In a synthetic tobacco line (*Nicotiana syvestris* x *N. tomentosiformes*), alterations in the structure of *N. tomentosiformis* rDNA units and the appearance of an extra locus in a *N. tomentosiformis* chromosome were observed (SKALICKÁ *et al.* 2003). These data were compared with those from an analysis of tobacco cultivars, indicating that an initial burst of rDNA evolution associated with allopolyploidy was followed by a slower process that led towards reduced complexity and a decreased number of rDNA variants.

In the case of the somatic hybrids of *Passiflora*, as they are female-sterile and pollen producers, further studies of a larger sample of somaclones would be interesting, to investigate if genetic stabilization occurred among some of these materials obtained by vegetative propagation. On the other, hand, such an investigation could contribute to the study of the evolution of polyploidy in the genus *Passiflora*.

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